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The goal of this research is to characterize the role(s) of extracellular slime and of the cell surface in adhesion and motility of aquatic gliding bacteria. Initial biochemical and rheological analyses of the slime of *Flexibacteria maritimus* and several of its adhesion/motility mutants are described. A theoretical analysis of the function of slime in a motility mechanism based on travelling transverse undulating waves is described. Inconsistencies in adhesion of *F. maritimus* led us to measure the cell surface hydrophobicity of this and selected other gliding bacteria and determine how adhesion and motility are affected by critical surface energy (CSE) of the substratum. Wild-type gliders are relatively hydrophobic; they adhere most tenaciously to low CSE surfaces. For several gliding bacteria and their adhesion-deficient mutants and revertants, cell surface proteins that directly contact glass surfaces have been vectorially radio-iodinated. Distinct, complex labelling patterns are described. We also report the isolation of two marine gliding bacteria, one of which produces a high molecular weight inhibitor of adhesion of the second.

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PROJECT GOALS:

1. Characterization of the extracellular slime of aquatic gliding bacteria, particularly those isolated from marine biofilms, by physiological, genetic, biochemical, immunological, and rheological approaches.

2. Determination of the mechanism(s) of slime function in adhesion and motility of these gliding bacteria.

3. Characterization of cell envelope adhesins of the aquatic gliding bacteria that produce little or no slime.

Accomplishments for Year 2:

I. Extracellular Slime of *Flexibacter maritimus*

We have begun biochemical analyses of the extracellular polymers of static and shake cultures of several strains of *F. maritimus*. Freeze-dried supernatants of static cultures were separated on a Biogel P2 column. Carbohydrate-containing material was eluted in two fractions of molecular weight ~5 and 80 kDa. The higher molecular weight fraction polymer(s) contains D-glucose, D-galactose, D-mannose ,L-rhamnose and an unknown sugar (ratio of 2 : 1.9 : 2.9 : 1 : 0.5). Shake culture supernatants contained a single high molecular weight carbohydrate peak. This material is now being analyzed by HPLC.

In contrast to the common wisdom that the bulk of slime is polysaccharide, we have found a carbohydrate:protein ratio of

static culture supernatants of ~1:3; that of shake cultures is ~1:15. SDS-PAGE analysis of the slime reveals a variety of 2 polypeptides ranging in molecular weight from ~26-175 kDa. We are currently determining whether any of these polypeptides may be glycosylated. Some of these polypeptides are likely to be the extracellular hydrolases produced by this bacterium. Others may function in adhesion and/or motility.

Work Plan for Year 3: During the next year, we expect to complete the biochemical and rheological (see section V) analysis of F. maritimus slime, including a comparison of the polysaccharides and polypeptides produced by adhesion and motility mutants grown in suspension and on agar. We will also determine whether our slime preps are contaminated with lipopolysaccharide from the bacterial outer membrane. Additionally, we expect to initiate NMR analysis of the slime in collaboration with Allen Bush of UMBC's Chemistry Dept. Finally, we hope to begin a molecular genetic analysis of F. maritimus slime.

II. Adhesion Studies:

Some of the aquatic gliding bacteria, both marine and fresh water, demonstrate reproducible adhesion and motility on standard microscope slides. This has not been the case for F. maritimus, leading us to examine the influence of substratum properties on behavior of these gliders. In collaboration with J. Bonaventura and D. Rittschof of the Duke University Marine Lab, we have made extensive observations of F. maritimus and several other gliders on microscope slides derivatized with silanes so as to obtain a range of critical surface energies (CSE). At one extreme is TriDecaFluoroSilane (TDF) which has a low CSE (i.e. extremely hydrophobic). Muffled glass slides (baked at 500°C) provide surfaces of high CSE (extremely hydrophilic). The chemical uniformity of the surfaces of these derivatized slides is currently being assessed by J. Wightman (Dept. of Chemistry, Virginia Tech).

Our observations lead to the generalization that adhesion is most tenacious on low CSE surfaces and very tenuous on substrata of high CSE. Cells quickly and irreversibly attached to TDF; adhesion was so tight that the bacteria were able to glide little if at all. In contrast, adhesion to muffled glass was weak, if it occurred at all. In some cases, it appeared that the gliders "skittered" on high CSE slides.

We quantitated adhesion to these derivatized slides by counting the density of adherent cells after submersion in a suspension of fixed bacterial concentration. While adhesion of F. maritimus cells correlated with our behavioral observations (ie. highest density on lowest CSE), there was no correlation between cell density and behavior for Cytophaga U67, a fresh water glider.

Work Plan for Year 3: We expect to use the results from these derivatized slide studies to quantitate adhesion and motility of

F. maritimus and its mutants, grown under conditions that result in variations in slime production. These studies will involve a tenacity-of-adhesion assay in which bacteria adherent to a rectangular cross-section capillary are subjected to a centrifugal forces or to regulated flow of medium.

III. Cell Surface Properties of Aquatic Gliding Bacteria

Recognition of the differences in adhesion/motility behaviors on substrata of varying CSE (section II) have lead us to examine the surface hydrophobicity of several species of gliding bacteria and selected non-adherent mutants. We have used several procedures to determine surface hydrophobicity including Rosenberg's bacterial adherence to hydrocarbon (BATH) assay, ammonium sulfate salting out, and hydrophobic interaction chromatography (HIC). In addition, H. Busscher (U. of Groningen) is currently examining some of our strains by Fourier transform IR spectroscopy.

F. maritimus grown in static culture has cell-associated slime that confers relatively hydrophilic properties on the bacteria; they partition into the aqueous phase in the BATH assay and pass through an octyl sepharose column. In contrast, shake culture-grown cells, in which extracellular polymers appear to be sheared off the bacterial surface, behave as though they are relatively hydrophobic. Thus, it appears that the bound slime confers hydrophilic properties on these marine bacteria.

Cytophaga U67, which appears to lack significant levels of extracellular slime, behaves as though it is relatively hydrophobic. Its adhesion-deficient mutants, selected by enrichment for bacteria that partition into the aqueous phase in the BATH procedure, are relatively hydrophilic.

IV. Iodination of Cell Surface Polypeptides that Contact Substrata

In this collaborative project with R.A. Bloodgood of the U. of Virginia School of Medicine, we have used an immobilized iodination catalyst (Iodo-Gen) to label those surface polypeptides of Cytophaga U67 that make contact with glass substrata. One or more of the ≥15 (!) polypeptides that are radio-iodinated is likely to be an adhesin in this strain. Some of these polypeptides have been localized to the cell envelope fraction; others may be periplasmic. The fact that integral cell envelope proteins are labelled suggests that what extracellular slime this bacterium may produce does not interfere with direct cell envelope contact with the substratum.

We have also compared the labelling pattern of the wild-type with that of three adhesion-deficient mutants and their revertants. We have found three categories of changes in these mutants:
1) appearance of new polypeptide species as detected by Coomassies blue staining and/or appearance of new iodinated polypeptides;
2) loss of labelled species that are iodinated in the wild-type;

3) apparent changes in migration of polypeptides in SDS-PAGE (always reflecting lower apparent molecular weight).
In the first case, the changes could be due to alterations in targeting of polypeptides to the cell surface and/or to changes in protein conformation resulting in increased accessibility to iodination. In the second case, the loss of labelling may be due to a decrease in the actual amount of polypeptide or to change in its conformation. The third case may reflect alterations in post-translational processing.

We have isolated several revertants of the adhesion-deficient mutants. Some of these revertants demonstrate a labelling pattern like that of the wild-type. However, one class of revertants of strain Adh2 demonstrates a unique radio-iodination pattern, unlike either parent or wild-type.

We are currently using vectorial iodination to label proteins on the surface of F. maritimus cells grown in static and shake culture. Preliminary results demonstrate qualitative and quantitative differences in iodination of cells grown under different culture conditions. It appears that cell-associated slime of static cultures blocks accessiblity of some cell envelope proteins to the substratum.

Work Plan for Year 3: We hope to identify those vectorially iodinated proteins that function as adhesins for F. maritimus and Cytophaga U67 and localize them in the cell envelope-slime complex.

V. Modeling of the Role of Slime in Gliding Motility

We have completed two parts of our development of a mathematical model for gliding motility driven by travelling waves of transverse undulations in the cell envelope:
1. by obtaining analytic solutions of the governing differential equations using a perturbation series in a small parameter; and
2. by writing computer codes for numerical computation using the method of finite differences.
The purpose for using two independent techniques for solving the mathematical problem is to establish the validity of the calculations (It is well known that computer codes and complex analytic methods can be wrong!). We are focussing on the numerical method because it can be used for a wider range of conditions.

In the calculations, we have assumed that the rheological properties of the slime can be represented by a model known as a third-order fluid. Presently, we are measuring the actual rheological properties of F. maritimus slime.

Work Plan for Year 3: Data from the rheological analyses will be used to establish representative rheological equations for this mathematical model of gliding motility.

VI. Inter-Specific Inhibition of Adhesion

In the course of seeking additional slime-producing marine gliding bacteria, we have isolated two benthic Cytophagales (not yet speciated) from a bio m in Buzzard's Bay, Massachusetts. These strains appear to be closely related based on physiology, morphology and % G+C of Chromosomal DNA (based on T_m). However, EcoR1 digests of the DNA of each strain provides some differences in banding profiles on agarose gels. And, the two colony types can be distinguished by striking differences in their iridescence that suggest differences in the cell surfaces of the two strains. We have just completed an experiment to identify cell surface proteins with the immobilized iodination protocol described in section IV. The strain that demonstrates red iridescence has a variety of polypeptides that make contact with glass, similar in complexity to Cytophaga U67. In contrast, the green strain has one clearly dominant ~45 kDa polypeptide that is iodinated. The results suggest that one protein dominates the surface of this strain.

Of particular interest to the goals of this research is the fact that the red strain produces a extracellular diffusible material that inhibits adhesion to and thus motility of the green strain on hydrophilic (only) substrata. The material is a heat-labile and protease-resistant macromolecule. Column chromatographic results suggest a molecular weight of > 200 kDa. We are currently fractionating concentrated culture supernatants on Biogel P2 to isolate this adhesion inhibitor.

Work Plan for Year 3: We propose to characterize the adhesion inhibitor and determine the range of bacteria against which it is active. We will also characterize the cell surface of the green strain. An independent source of funds will be sought to support this research.

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4. Burchard, R.P. 1989. Adhesion of Gliding Bacteria. Invited presentation to the 1989 Gordon Conference on The Science of Adhesion.

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6. Burchard, R.P., D. Rittschof and J. Bonaventura (in preparation). Adhesion and motility of gliding bacteria on substrata of different surface free energies.

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